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Rapid Identification of *Staphylococcus aureus* from Blood Culture Bottles by a Classic 2-Hour Tube Coagulase Test

CLIFFORD L. McDONALD[1]† AND KIMBERLE CHAPIN[2]*

Division of Infectious Disease, Department of Medicine,[1] and Department of Pathology,[2] University of South Alabama, Mobile, Alabama

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The rapid, reliable identification of *Staphylococcus aureus* from positive blood cultures provides important information. While multiple methodologies for detection of *S. aureus* from blood culture broths exist, none is satisfactory. Immunologic tests have shown varied sensitivities, the thermonuclease test, while sensitive, is not practical for routine use, and probe tests are expensive. Few studies have addressed using the tube coagulase test (TCT). This study compared two immunologic methods, the Staph Latex kit (Remel Laboratories) and the Staphaurex kit (Wellcome Diagnostics), with a rabbit plasma TCT (Difco Laboratories) to identify *S. aureus* within 2 h directly from blood culture broths and pelleted supernatants from BACTEC (Johnston Laboratories) bottles. One hundred twelve unique clinical blood culture isolates consistent with a Gram stain for staphylococci and 68 negative blood culture bottles seeded with a variety of gram-positive organisms were evaluated. Sensitivity and specificity among clinical specimens for the 2-h TCT were 79.5 and 100%, respectively. Sensitivities for the immunologic methods were 12.8 and 10.2% for the Staphaurex and Remel Staph Latex, respectively, and specificities for both were 100%. These results contradict previously reported results for both immunologic and TCT methods and dictate that a specific as well as a sensitive method be employed. The 2-h TCT was found to be a cost-effective, reliable, and rapid method for identifying *S. aureus* from positive blood cultures.

The rapid, reliable identification of *Staphylococcus aureus* from clinical specimens is important given the difference in virulence between *S. aureus* and coagulase-negative staphylococci as well as the relatively high incidence of coagulase-negative staphylococci isolated as a contaminant. This is especially significant in the rapid identification of gram-positive cocci from blood cultures when test results can determine the need for immediate therapy.

The traditional method of differentiating staphylococci in the clinical laboratory has been identification of extracellular coagulase production by *S. aureus* using a tube coagulase test (TCT). Typically, rabbit plasma is inoculated with growth from isolated colonies and examined for the presence of a gel or clot at 4 h and, if negative, examined again at 24 h. Alternative and faster methods for identifying *S. aureus* have evolved, including the slide coagulase test, rapid thermonuclease test, DNA probe, and multiple commercial kits that use antibody-coated latex particles for the detection of cell-associated clumping factor and/or protein A produced by *S. aureus*. Most of these tests are convenient and fast while maintaining accuracy in most applications (1). In addition, several investigators have evaluated the accuracy of the agglutination tests in rapid identification of *S. aureus* from positive blood culture broths (3–5). Although overall specificity has been excellent, a wide range of sensitivities have been reported for a variety of latex tests. Because our laboratory found sensitivities below that reported in the literature for rapid latex tests from blood culture pellets and the staphylococcus latex test currently used in our laboratory had not been previously evaluated, the accuracies of latex tests and the TCT for the rapid detection of *S. aureus* directly from positive blood culture broths as well as pellets generated from a blood culture supernatant after centrifugation were assessed.

* Corresponding author. Mailing address: Department of Pathology, University of South Alabama Medical Center, 2451 Fillingim St., Mobile, AL 36617-2293. Phone: (205) 471-7783. Fax: (205) 471-7884.

† Present address: Department of Clinical Microbiology, Duke University Hospital, Durham, NC 27710.

MATERIALS AND METHODS

The Staphaurex (Wellcome Diagnostics; Dartford, England) test, which has shown the best sensitivity for detection of *S. aureus* from blood culture broths in previous studies (4, 5), and the Staph Latex kit (Remel Laboratories; Lenexa, Kans.), the test currently used in the University of South Alabama clinical laboratories, were evaluated and compared with a 2-h TCT using rabbit EDTA plasma (Difco Laboratories; Detroit, Mich.). Sixty-eight seeded and 112 clinical specimens were isolated from BACTEC 6A (nonradiometric) and 7A (nonradiometric) blood culture bottles (BBL, Towson, Md.).

Seeded blood cultures. Seeded blood cultures were made by a method comparable to that of Doern and Robbie (3). Five-day-old negative blood culture bottles were inoculated with broths of test strains obtained from clinical specimens processed in the laboratory with a variety of gram-positive cocci identified by conventional criteria. A suspension equivalent to an 0.5 McFarland turbidity standard was prepared in sterile saline, and subsequently, a 10^6 dilution was made. Aerobic and anaerobic blood culture bottles containing approximately 4 ml of blood were inoculated with 0.1 ml of the organism suspension, such that bottles contained 3 to 15 CFU per bottle and resulted in a growth index range of 30 to 150 at 24 h. All bottles inoculated were deemed positive within 24 h, and coagulase tests were performed.

Clinical blood cultures. Clinical blood cultures were processed according to conventional criteria. Three to five milliliters was inoculated into each bottle of an aerobic-anaerobic set. Aerobic blood culture bottles were agitated and incubated at 37°C, while anaerobic bottles were incubated at the same temperature without agitation. All cultures were read twice daily for the first two days and once daily thereafter for a total of 5 days on the BACTEC 660 system. Any bottle that yielded a growth index of ≥30 within the first 48 h from placement was removed, and a Gram stain was performed. Any broth that yielded a Gram stain consistent with *Staphylococcus* spp. was tested by all three coagulase test methods.

Positive blood broth processing. A modification of the method of Rappaport et al. (5) was used in processing the blood cultures for rapid identification. The procedure, in brief, was as follows. Ten milliliters of the medium was aseptically removed from the bottles, and 3 drops were used for each of the three coagulase tests. The remaining medium was centrifuged at 150 × *g* for 10 min, and the supernatant was transferred to a second tube. The supernatant was then centrifuged at 1,000 × *g* for 15 min, the pellet was resuspended in 1 ml of 0.85% sterile saline, and a 3-drop sample was tested with each of the three coagulase tests.

TABLE 1. Results of coagulase tests with seeded and clinical specimens

Culture and organism(s)	No. aerobic/no. anaerobic (total)[a]	Mean growth index[b]	No. of positive results					
			Direct[c]			Pellet[d]		
			Staphaurex	Remel	TCT	Staphaurex	Remel	TCT
Seeded								
Staphylococcus aureus	11/8 (19)	81	0	0	19	2	2	19
Coagulase-negative staphylococci	9/9 (18)	78	0	0	0	0	0	0
Staphylococcus pneumoniae	5/5 (10)	86	0	0	0	0	0	0
Enterococcus spp.	6/5 (11)	60	5	5	0	5	5	0
Streptococcus agalactiae	3/5 (8)	82	0	0	0	0	0	0
Streptococcus pyogenes	1/1 (2)	36	0	0	0	0	0	0
Clinical								
Coagulase-negative staphylococci	70/3 (73)	82	0	0	0	0	0	0
Staphylococcus aureus	35/4 (39)	100	5	4	31	5	4	30

[a] Of the 68 seeded cultures examined 35 were aerobic and 33 were anaerobic. Of the 112 clinical cultures examined 105 were aerobic and 7 were anaerobic.
[b] Averages for seeded and clinical cultures, 76.6 and 88, respectively.
[c] Respective totals for seeded and clinical cultures: Staphaurex, 5 and 5; Remel, 5 and 4; TCT, 19 and 31.
[d] Respective totals for seeded and clinical cultures: staphaurex, 7 and 5; Remel, 7 and 4; TCT, 19 and 30.

Coagulase tests. Both latex tests were performed according to the manufacturer's instructions. The TCT was performed with glass tubes and incubation at 37°C for 2 h, and the results were scored as a positive clot or no clot. If the 2-h reading was negative, the tubes were then left at 25°C for up to 24 h and read again for clot formation.

RESULTS

The sixty-eight negative blood cultures seeded with gram-positive organisms resulted in a mean growth index of 76.7 (standard deviation, 25.4) and a range of 34 to 149 at 24 h. The number of bottles seeded with different organisms, types of bottles used (aerobic and anaerobic), and the results of rapid identification tests for both the broth culture and the pellet are listed in Table 1. Among the 68 seeded blood culture bottles, the 2-h TCT correctly identified 19 of 19 blood cultures seeded with 12 different *S. aureus* isolates. Neither latex test correctly identified any of the *S. aureus*-seeded bottles. There were no false positives with the 2-h TCT, while both latex tests gave false-positive results for 5 of 58 non-*S. aureus* isolates. All false positives were from bottles seeded with enterococci. There was no difference between the performance of the 2-h TCT directly on the blood culture fluid and its performance on a pelleted supernatant after an initial centrifugation.

The results for 112 clinical specimens tested with the three rapid identification methods are also listed in Table 1. The mean growth index was 88.3 (standard deviation, 34.7) with a range of 30 to 146. Among these clinical specimens, the 2-h TCT correctly identified 31 and 30 of 39 *S. aureus* isolates directly in blood culture fluid and in pelleted supernatants, for sensitivities of 79.5 and 76.9%, respectively (Table 2). The Remel Staph Latex and Staphaurex tests identified only four and five of these isolates, respectively, using either direct or pelleted material, resulting in sensitivities of only 10.2 and 12.8%, respectively. Specificity with clinical isolates was 100% for all methods tested.

Data were pooled from both the seeded specimens and the clinical specimens. The mean growth index associated with false-negative results for the 2-h TCT was 103.8 compared with a mean growth index of 91.9 among correctly identified positives. This difference in growth values was not statistically significant ($P = 0.28$ [independent t test]). Overall, sensitivities for the TCT were 86.2 and 84.4% for a direct blood culture broth and pelleted broth, respectively (Table 2). Specificity for the TCT was 100%. Overall, sensitivities for the Staphaurex and Remel Latex tests were 8.6 and 6.8%, respectively, and specificity was 95.9% for both. Evaluation of the TCT at 4 and 24 h did not identify additional isolates as positive.

DISCUSSION

Our data suggest that two commonly used latex kits are neither sensitive nor specific when used to rapidly identify gram-positive cocci from positive BACTEC blood culture broths. These data contradict several previously published trials of latex kits used for this purpose. Doern and Robbie (3) evaluated a commercial latex agglutination slide test (Sero-STAT Staph, Scott Laboratories) to rapidly identify *S. aureus* from both seeded and clinical radiometric BACTEC blood culture bottles. The procedure used involved two successive centrifugations at a high speed with washing of the pellet between centrifugation steps. Using this methodology the investigators were able to achieve a 100% accuracy for 30 seeded cultures and a 94% accuracy for 36 clinical specimens.

Rappaport and colleagues (5) later compared multiple immunologic identification methods for the rapid identification of *S. aureus* from blood culture bottles and found a range of sensitivities from 38.6% for the Staphyloslide kit (BBL) to 77.3% for the Staphaurex kit. No false positives were found with any of the immunologic methods tested, but only catalase-positive gram-positive cocci were tested. To perform the testing, an initial slow-centrifugation step to precipitate eryth-

TABLE 2. Results of TCT and immunologic tests

Cultures and test[a]	Sensitivity (%)		Specificity (%)	PPV[b] (%)
	Direct	Pellet		
Clinical (112)				
Staphaurex	12.8	12.8	100	100
Remel	10.2	10.2	100	100
TCT	79.5	76.9	100	100
Clinical and seeded (180)				
Staphaurex	8.6	8.6	95.9	50.0
Remel	6.8	6.8	95.9	44.4
TCT	86.2	84.4	100	100

[a] The total numbers of specimens are given in parentheses.
[b] PPV, positive predictive value.

rocytes and debris was followed by a fast-centrifugation step to pellet bacteria. When we performed catalase tests either on the supernatant of the first-centrifugation step or on the pellet of the second centrifugation, an unacceptably high incidence of false-positive results, especially among anaerobic bottles, was found. This was presumably due to retained blood components which were catalase positive. Thus, it appeared that a reliable, rapid identification method for the differentiation of staphylococci from streptococci other than Gram stain morphology did not exist. Therefore nonstaphylococcus gram-positive organisms were included among our seeded cultures, as these might occasionally be misidentified by blood culture Gram stains.

We also compared the preparation method used by Doern and Robbie (3) with the method used by Rappaport et al. (5) and found no difference in results with seeded blood culture bottles. Since the method of Rappaport et al. was simpler to use, we chose this method for our study.

More recently, Davis et al. (2) reevaluated the rapid identification of *S. aureus* directly from blood cultures. To do this, they performed two immunologic tests and the TCT on drops taken directly from the blood culture broth and drops from the pellet obtained after the second centrifugation step by the method of Rappaport et al. (5). Using the Staphaurex kit and the Staphyloslide kit on blood culture pellets, the investigators found sensitivities of 38 and 33%, respectively, with a specificity of 98% for each test. The TCT read at 2 h, however, performed much better with a sensitivity and specificity of 100% when used directly on 71 positive clinical blood cultures. Although they tested only a few isolates, these authors found that both the Staphaurex kit and the Staphyloslide kit performed better when tested directly on blood culture broth, with a sensitivity of 80% and specificity of 92% for both tests. They went on to demonstrate a sensitivity of 97% and a specificity of 100% with the 2-h TCT performed directly on the broth for a total of 189 clinical blood cultures which were positive for *Staphylococcus* species on the basis of Gram stain morphology.

In our study the Remel Staph Latex kit and the Staphaurex kit both performed poorly. In fact, these are the poorest results to date for using the Staphaurex kit for this purpose. These results and the overall consensus from the literature suggest that latex kits should not be relied upon for the direct identification of staphylococci from blood culture bottles. Our results are somewhat unique in being the first to report the false positives that may occur if enterococci from blood cultures are inadvertently misidentified by Gram staining as staphylococci and tested with these kits. These false positives did not occur with the 2-h TCT.

Although the 2-h TCT used either directly on the blood culture broth or on the pellet had an acceptable sensitivity as well as excellent specificity and positive predictive value, the sensitivity results were not as good as those reported by Davis et al. (2). One possible reason for this is that we studied the clinical isolates as part of our normal clinical microbiology work flow. This meant that a variety of technologists who rotated on the blood bench over the period of this study read the latex kit and TCT results. Since the interpretation of weakly positive results of these tests is very user dependent, we felt that performing this study with multiple participants would more accurately predict sensitivity and specificity when these tests are used routinely in the clinical microbiology laboratory. These results may also have differed because rabbit plasma from different manufacturers was used in other than glass tubes.

Another method of identifying *S. aureus*, the thermonuclease test, which differentiates *S. aureus* on the basis of the ability of the DNase it produces to withstand heating, has also been successfully applied to rapid identification from positive blood cultures. Although a recent study of the use of this method reported a sensitivity of 95% and specificity of 100% (6), the method involves a boiling step, making it inherently more complicated to perform than either the latex test or the TCT.

The costs of the TCT, latex tests, and thermonuclease test are approximately equivalent at approximately $1.00 each, including a positive control with each test.

The use of the TCT read at 2 h for the rapid, direct identification of staphylococci from positive blood culture broths is a return to a faithful standard (7). In addition to being simple to perform and easily adapted into the laboratory work flow and having demonstrated positive predictive value, it is relatively inexpensive. With the modification of reading the result at 2 h and using blood culture broth directly, the ease of the TCT is comparable to the convenience of the latex test. Given the moderate sensitivity (86.2%) of the 2-h TCT, negative results should be reported as "staphylococcus, full identification to follow," while positive results may be confidently reported as *S. aureus.*

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